Understanding Why Researchers Should Use Synchrotron-Enhanced FTIR Instead of Traditional FTIR

Michelle R. Stem
Department of Materials Science and Engineering, The University of Texas at El Paso, El Paso, TX 79968; *mrstem@utep.edu*

Synchrotron-enhanced Fourier transform infrared (SR-FTIR) is a relatively new method of combining a synchrotron's unrivaled brightness, power, focusability, polarization, and tunability with the well-established analytical approach of a traditional FTIR. Although SR-FTIR photons range from infrared (IR) through soft X-ray, applications and discussions herein are limited to the IR range of the spectrum. A SR-FTIR system detects, interacts with, and examines molecular species that respond to IR photons (IR responsive). Thus, SR-FTIR is able to examine only the lighter elements (i.e., C, N, O, etc.), which are the most common elements found on earth. The ability to study these elements using the superior abilities of SR-FTIR, versus traditional FTIR, is transforming scientific understanding of many species interactions (i.e., biological, organic, etc.). Furthermore, the lighter elements are invisible to the only other synchrotron (SR) category, a relativistic heavy ion collider (RHIC or, colloquially, an atom smasher), which detects heavier elements that are invisible to a SR-FTIR (i.e., Ta, Pt, Au, etc.) *(1a, 2, 3a,b, 4a,b, 5a,b, 6–9)*.

FTIR Spectroscopy Overview

At temperatures above absolute zero, every atom of every molecule vibrates ceaselessly. Generally, the combination of a various vibrational frequencies results in a molecule's unique characteristic pattern (fingerprint). A molecule absorbs IR radiation when the vibration of the atoms in the molecule produces an oscillating electric field with the same frequency as that of incident IR photons. Molecules will have FTIR fingerprints, provided the molecule is IR-responsive. A molecule is considered to be IR responsive only when the absorption of an IR photon causes at least one of the atoms to change its vibrational state and the molecule to change its dipole moment. Thus, FTIR has limits because it will not detect an organic molecule with structures symmetric about a bond of interest. For example, the bonds between carbon atoms of ethane are not IR-responsive because there is a methyl group at each end of the bond. Yet, the C−H bonds of the methyl groups are IR-responsive because changing their vibration alters the dipole moment of ethane *(1a, 10)*.

SR-FTIR Versus Traditional FTIR

A SR-FTIR system can be used to precisely identify specific molecules, atoms, and bonds of IR-responsive species because a SR-FTIR reveals IR-responsive fingerprints at a level of detail that is unrivaled by traditional FTIR. Experiments indicate that SR-FTIR is better than traditional FTIR at revealing detailed spectra necessary to identify a sample's constituent species, especially when analyzing systems that are organic, biological, complex, dilute, or living. For example, common biomolecules, such as nucleic acids, proteins, and lipids are IR-responsive *(4a, 10, 11)*.

Only SR-FTIR allows researchers to discriminate the minuscule energy differences of particular bonds by controlling and focusing the photons used for analyses. Thus, researchers are able to precisely identify bonds and changes in bonds in dilute samples and over time (i.e., during a reaction). SR-FTIR is able to monitor in-process chemical changes via time-resolved experiments. SR-FTIR microspectroscopy is becoming an analytical tool of choice for its ability to probe non-destructively biological, organic, and even living-cell species. The distribution of chemical species in heterogeneous samples can be investigated by SR-FTIR microspectroscopy mapping and imaging, wherein even fast reactions or very small, dilute, or scarce samples can be measured and mapped at spatial resolutions of at least 10 μm (spot size is diffraction limited) and hundreds of times brighter than traditional FTIR. Traditional FTIR microspectroscopy is also limited by a low signal-to-noise ratio that masks signals and limits resolution. Additionally, molecular exposure to traditional FTIR sources has been shown to cause bond breaking, ionization, and other damage *(1b, 5b, 11–16)*.

Photons from traditional FTIRs have such high beam divergences that they have spot sizes of about 100 μm, and a flux loss that is at least two orders of magnitude greater than that of SR-FTIR. Although traditional FTIR sources emit total photon fluxes comparable to that of SR-FTIR, SR-FTIRs emit photons that have virtually no beam divergence because they are collimated and coherent (spot size ≤ 3 μm). Moreover, even though a SR-FTIR is very bright, a SR-FTIR system causes a sample's temperature to rise very little (0.5 ± 0.2 K). Even if a sample is exposed for days, SR-FTIR exposure will not alter living cells, organic, biological, and other species *(1b,c, 5b, 11, 12, 15, 16)*.

Main Components of SR-FTIR

Electron Gun and Linear Accelerator

As indicated in Figure 1, the SR-FTIR's action starts with an electron gun emission of a thin stream of electrons, which are then attracted towards a linear accelerator (LINAC). A LINAC unit transforms the stream into a flow of 500 million loose bunches of electrons per second (500 MHz), culminating in the LINAC forming the loose electron bunches into highly uniform, tight packets traveling at relativistic velocities before being propelled into a booster ring *(2a, 3a,b, 17, 18)*.

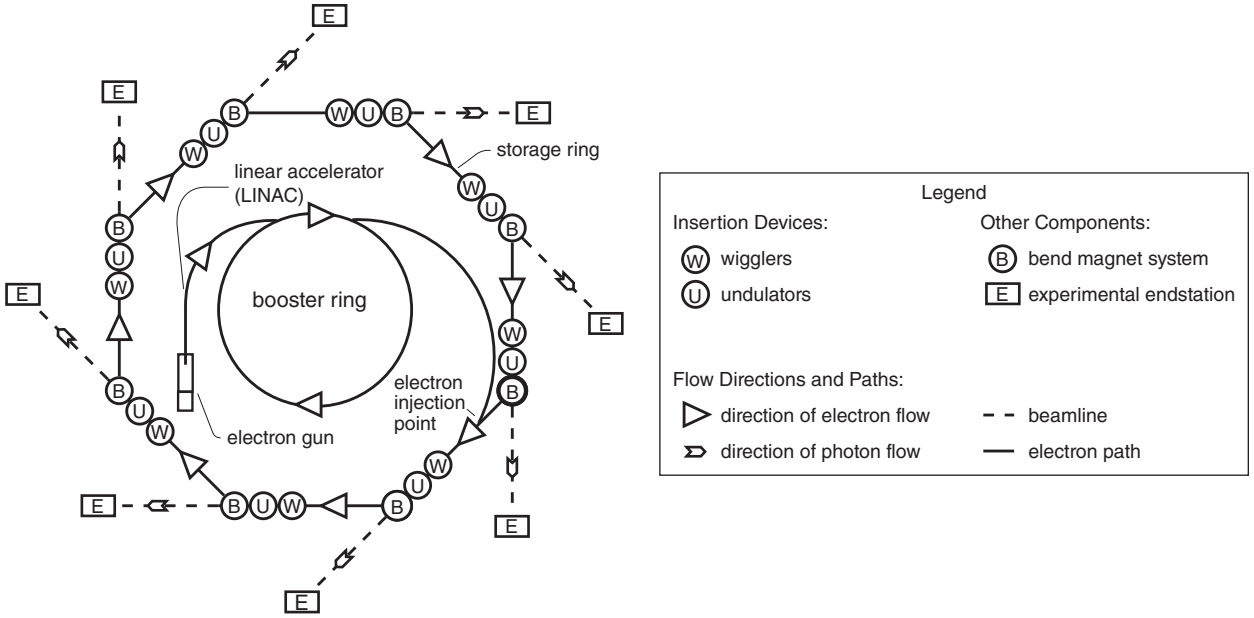

Figure 1. Key components of the SR-FTIR.

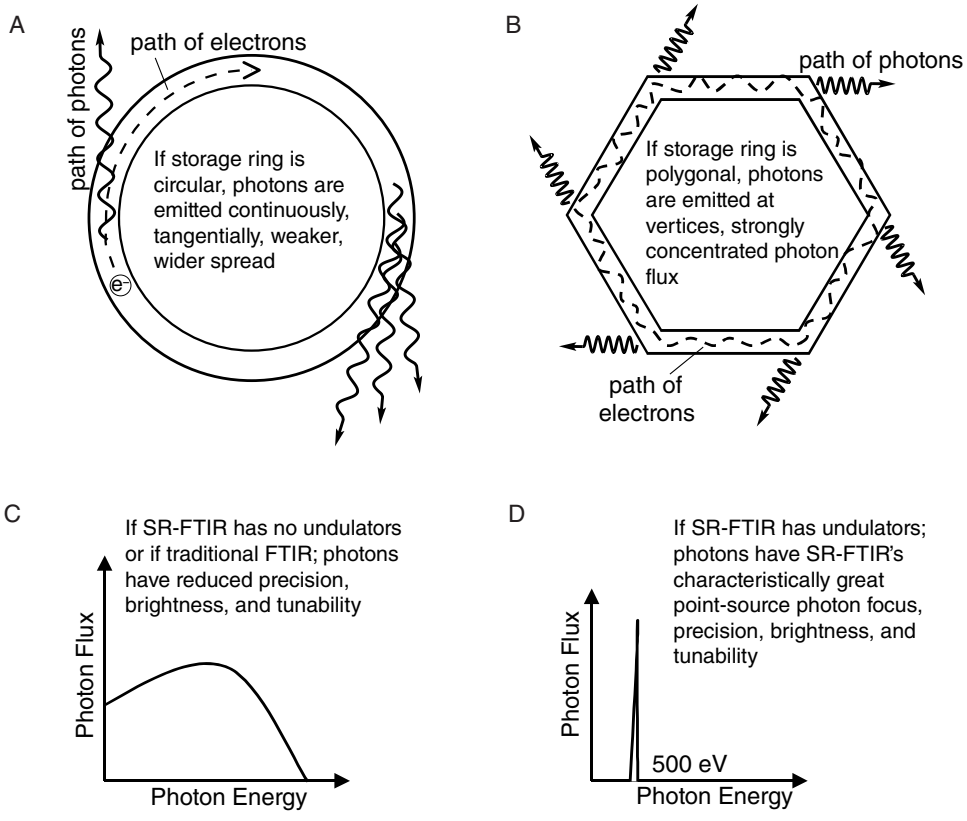

Figure 2. Effects on emitted SR electron packets and resulting photon packets: (A, C) without undulators and (B, D) with undulators (*1d*, loosely adapted, used with permission)

Booster Ring

The booster ring maintains the velocity of each electron packet while increasing the packets energy by at least an order of magnitude (e.g., from about 12 MeV to 2.5 GeV). In effect, the booster ring is a space-saving circular LINAC. A booster ring is used in SR-FTIR facilities such as Advanced Light Source (ALS; Berkeley National Laboratory, CA), National Synchrotron Light Source (Brookhaven National Laboratory, NY) and ELETTRA (National Laboratory, Italy). In less than one second, an electron packet in the booster ring reaches 99.999994% of the speed of light, completes more than a million revolutions, and reaches its target energy before being discharged into the storage ring *(2a,b, 3a,b, 17, 18)*.

Although the majority of SR-FTIR facilities, such as the Center for the Advancement of Natural Discoveries using light emission (CANDLE) *(18),* the Stanford Synchrotron Research Labs (SSRL) *(2b),* and the ALS *(1a)* use one booster and one storage ring (although SSRL also has a RHIC at the same facility). At least one SR-FTIR facility, ELETTRA *(3b),* uses no booster ring. Yet, each SR-FTIR facility is capable of comparable research *(3a, 17)*.

Storage Ring

The storage ring is largest component of the SR-FTIR, with diameters ranging from about 35 m to 220 m, depending on the facility. A storage ring is composed of many straight sections of ultra-high vacuum tubes, assembled into a large polyhedron, where each vertex is called a "bend". Electron packets revolve in the storage ring more than a million times per second, emitting an intense photon flux when each packet traverses a bend. After a bend, the SR uses magnet systems and klystrons to recharge the electron packets before the next bend *(1a, 2a,b, 3a,b, 5a, 17, 18)*.

Magnet Systems

Primarily, the SR-FTIR magnet system enables emission of the typically bright, coherent, polarized photon beams. The parameters of the magnet system can be finely tuned to alter the properties of the circulating electron packets, hence controlling photon traits. Bend, undulators, and wigglers comprise the main magnet systems. Undulators and wigglers are also called insertion devices. Insertion devices allow electron packets to emit SR photons (Figure 2B and 2D). This contrasts with Figures 2A and 2C, indicating broad unfocused photon energy that is neither monochromatic nor energy specific, owing to a lack of insertion devices *(1a,b, 2b, 3a–c, 4b, 5b, 12, 17)*.

Bend Magnet Systems

The effect of bend magnets is somewhat analogous to a race car on a dirt track. A race car engine generates a boost of acceleration to help it accelerate to a maximum speed before each bend, control the car's speed and direction around each bend, and then prepare for the next bend. At each bend, a boost is needed because the race car slows down, losing power that is diverted to the tires' spraying dirt (photons) tangentially to the car's path of travel (Figures 2C and 2D). Bend magnet systems control and propel electron packets past bends, at which vertices they emit photon pulses (Figures 1 and 2B) *(1a,d, 3a–c, 4a, 5a,b, 17)*.

Undulator Insertion Device

A main contributor to the superiority of SR-FTIR over traditional FTIR is undulators. Comparing SR photon emissions without and with undulators is similar to comparing a floodlight with a laser (Figures 2C and 2D). Undulators cause photon emissions that are the brightest photon beams made by humans and are at least 10^3 brighter than would be produced by bend magnets alone *(3a–c, 12, 17, 19)*.

Undulators apply precisely periodic magnetic fields that force the electron packets to follow a planar zigzag pattern (Figure 2B) and generate point-source energy (Figure 2D). Undulators have a series of magnets with alternating polarities along the storage ring straight sections (Figure 1). When passing a bend, each electron packet emits an intense photon pulse that constructively interferes with each prior photon pulse. These photon pulses additively overlap each other into seamless SR photon beams that are directed along a beamline. Adjusting the undulators (i.e., position, polarity, etc.) changes the beams' traits (i.e., flux, brightness, wavelength, etc.) *(1d, 3b,c, 12, 19)*.

Wiggler Insertion Device

SR-FTIR wigglers are mechanically similar to undulators. However, wigglers emit photon pulses, not beams, along a beamline by causing electron packets to make small, sharp planar zigzags. The packets emit broadband photon pulses, with minimal overlap at the extremum of each zigzag, that are at least 100 times brighter than are those from bend magnets alone. Wiggler pulses enable sample scanning across IR wavelengths with almost no background noise. Moreover, wigglers can make precision biological and organic electron density maps of living systems, including cells *(3b,c, 4a, 17)*.

Beamlines and Experiment Endstations

The SR-FTIR photon beam is guided from the storage ring through meters of high-vacuum pipe, called a beamline, to an experiment endstation. Some experiments use almost all of a beamline's available photons (i.e., time-of-flight). Others expose samples to only very specific photon energies (i.e., seeking a particular bond), a capability that traditional FTIRs lack. *(1b,c, 2b, 3a,c)*

Brightness

A candle is about five orders of magnitude dimmer than the sun. The brightness difference makes it apparent that a candle and the sun have drastic photon flux differences. Consider the following; the photons emitted from a SR-FTIR system without undulators are at least five orders of magnitude brighter than the sun and about eight orders of magnitude brighter than traditional IR sources. Moreover, the photons emitted from a SR-FTIR system with undulators are about four orders of magnitude brighter than a SR-FTIR system without undulators. A SR produces the brightest light known and undulators cause the brightest SR light, its photons being concentrated into a beam ≤ 3 μm in diameter. Coupled with its nearly silent background noise, a SR-FTIR is able to analyze IR-responsive species by applying far more concentrated flux and brightness and hence revealing details unable to be seen by traditional FTIR *(1b, 3b, 5a)*.

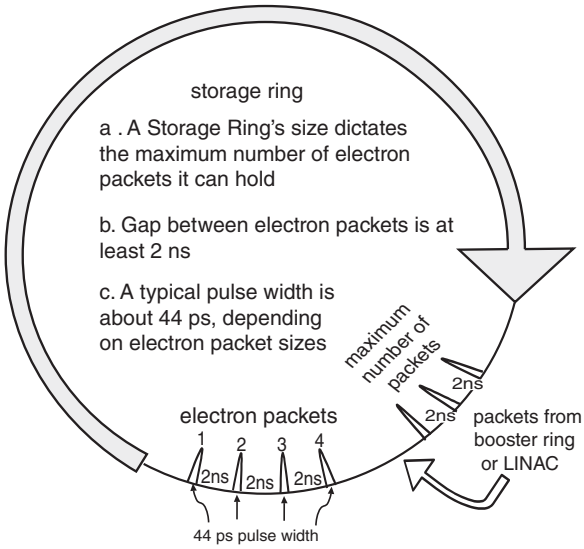

Figure 3. SR-FTIR electron packets are carefully pulsed and spaced, and then revolve in a storage ring. Figure is indicative only. (*1c*, loosely adapted, used with permission)

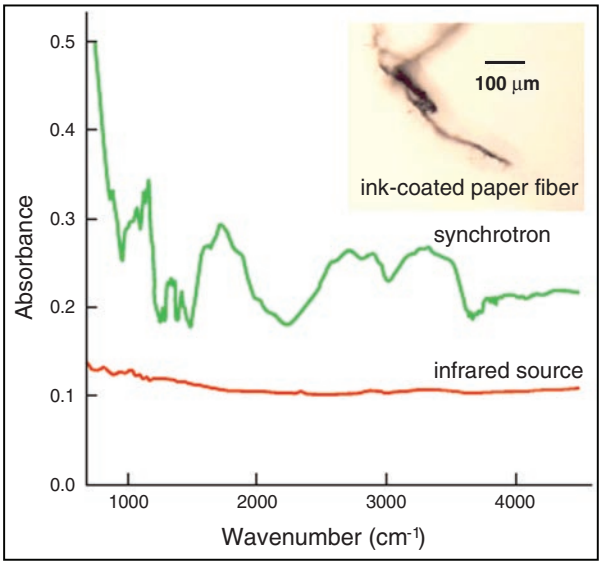

Figure 4. The same ink-coated paper fiber scanned with SR-FTIR (top line) and traditional FTIR (bottom line) (*23*, used with permission).

Time Resolution

Figure 3 indicates that controlling SR electron packets enables time-resolved FTIR spectroscopy because the pertinent properties of SR-FTIR photons are highly sensitive to adjustments of electron-packet size, the total number of packets in the storage ring, packet energy, and packet spacing. SR-FTIR adjustable photon properties, which enable extremely sensitive time resolution, include the precision setting of pulse time gaps (in ps increments), photon wavelengths, pulse frequencies, and pulse durations. The maximum number of electron packets that can revolve in the storage ring at any one time (average ≈ 350) is based on the storage-ring size, with each separated by about 2 ns. The storage ring can hold any combination or size of electron packets, from 1 to its maximum. Thus, taking sample images at specified intervals or for specified durations (i.e., pump-probe) is possible with great precision differentiation between minuscule time increments. Thus, three-dimensional resolution of samples and fast reactions of chemical and biological systems are able to be studied in fine detail. Traditional FTIR facilities lack sufficient flux and pulse control exactitude and so are unprepared to do SR-FTIR level of time-resolved or three-dimensional measurements *(1b,c, 3a–c, 4c, 5b)*.

Applications Summary

SR-FTIRs reveal fine molecular-vibration details about the chemistry and structure of IR-responsive materials, yet without destroying or altering bonds, atoms, or molecules, even for living samples. This contrasts with the rampant bond breakage caused by traditional FTIRs. Further, SR-FTIRs have been able to monitor the chemistry within living cells without using chemical tags, which traditional FTIRs cannot do. Additionally, combining a SR-FTIR with microspectroscopy makes a powerful tool with a level of analytical ability that a traditional FTIR cannot match. Specifically, SR-FTIR microspectroscopy non-destructively probes and images small biosystems, individual living cells, cellular colonies, materials, and heterogeneous biological systems. SR-FTIR displays signal peaks with rich character detail, while traditional FTIR would lose signal peaks behind background noise. SR-FTIR operates optimally at frequencies that include the IR-responsive fingerprint range of biomolecular species (4000–650 cm^{-1}), such as bonds within nucleic acid helices (i.e., DNA) *(3a, 4c, 13–15, 20, 21)*.

SR-FTIR has shown itself to be superior, compared to traditional FTIR, in studying a diverse array of organic and biological samples. SR-FTIR applications include agriculture, biochemistry, biological, blood, cellular, chemical kinetics, documents, drugs, engineering, environmental, explosives, fibers, forensics, geology, inks, laminates, materials, microtechnology, nanoscience, paints, particulates, polymers, powders, proteins, semiconductors, structures, surfaces, and thin films. SR-FTIR is playing an increasingly significant role in forensics because of its repeatedly verified unique ability to reliably and non-destructively examine a variety of IR-responsive samples in superlative detail *(1a–c, 3a, 4b,c, 13–15, 17, 20–23)*.

Cost and Access

Access Fees

SR-FTIR facilities typically charge academic researchers a token fee for beamline time (and nominal fees for supplies). Industrial researchers are typically charged higher fees for beamline time *(3a, 5a, 18, 23)*.

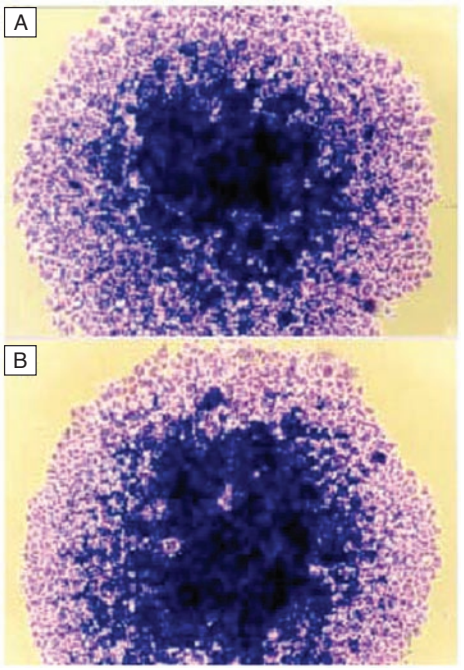

Figure 5. Two human T-1 kidney cell colonies are compared, showing that the colony exposed to SR-FTIR photons had no damage (*16*, used with permission).

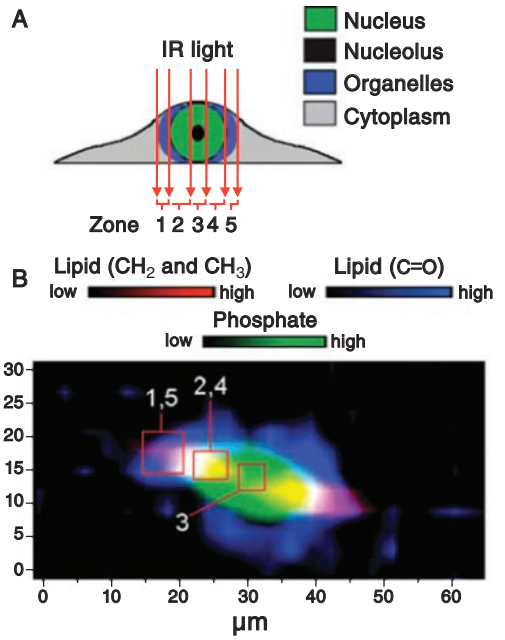

Figure 6. SR-FTIR microspectroscopy study of a living prostate cancer PC3 cell reveals tremendous details (*17*, used with permission). (Part B is shown in color on page 885.)

Building Costs

The extensive funds to build and operate the 50+ SR-FTIR facilities present worldwide have primarily come from a combination of governments, both local and through multinational cooperation. Building a SR-FTIR would be too costly for most universities. Yet, a few universities have SR-FTIRs, for instance Monash and Berkeley Universities. Specifically, the anticipated construction cost for the Australian synchrotron is $210 million by completion year 2007 *(3a, 4d, 5a, 18, 23)*.

Access

Almost all SR-FTIR facilities receive more requests to use their facilities than can be scheduled in a given time period. Hence, facilities require potential researchers to submit a research proposal that explains the value of their intended experiment and anticipated needs (time, supplies, beamline, etc.). Each SR facility schedules beamline time for those proposals that meet the facility's valuation criteria *(1e, 3b, 12)*.

Case Studies

Figure 4 compares SR-FTIR versus traditional FTIR scans of the same paper fiber. The SR-FTIR scan (top line of Figure 4) shows fine IR fingerprint character of the ink. The SR-FTIR scan line indicates, from the right to the left: OH stretch (2500–3300 cm^{-1}), carbonyl stretch (1820–1660 cm^{-1}), and the small peaks at 1500 cm^{-1}, which include alkene bending (1650–650 cm^{-1}), CH_2 bend (1450 cm^{-1}), CH_3 bend (1400 cm^{-1}), and CO single bond stretch (1200 cm^{-1}). However, the traditional FTIR scan (bottom line of Figure 4) erroneously indicates that nothing IR-responsive was present in the sample *(20)*.

The SR-FTIR background noise is virtually silent, causing almost no competitive blocking of sample signals. However, in this study, traditional FTIR sources have so much background noise that they can obscure valid sample signals, whereas SR-FTIR has a lack of sample heterogeneity problems. Also, this study shows that SR-FTIR can produce IR scans with tremendous details from minute samples and that minutes of constant SR-FTIR exposure do not alter chemical structures *(1b, 3a, 11, 18, 20)*.

Figures 5A and 5B show two healthy colonies of living T-1 kidney cells: 5A is a control colony and 5B is a test colony that was exposed to SR-FTIR photons for 20 minutes. After exposure, both colonies proliferated identically for all 10 days of the study, including all aspects of cellular health. Although the results by Holman et al. (Figures 5A and 5B) revealed no evidence that SR-FTIR exposure had any cytotoxic or other effects on the colonies, they are indicative only *(3a, 4c, 13, 16)*.

Figures 6A and 6B show a SR-FTIR microspectroscopy analysis map and image of a single living prostate cancer PC3 cell. Figure 6A shows the PC3 cell's chemical contents mapped into diagrammed zones. Figure 6B is a SR-FTIR microspectroscopy image of an unharmed live PC3 cell from which the map was derived. The image was made by moving the SR-FTIR detector over the cell in aperture-sized increments (at least ≥ 5 μm). At each increment, the SR-FTIR took an IR transmission image through the living cell, spot-by-spot, until the image was complete. Differences in IR transmission rates indicated the volume, location, and presence of various PC3 cellular components. The cell's more prominent peaks were lipid acyl chain (≈ 2840–2794 cm^{-1}), amide I (≈ 1645 cm^{-1}), phosphate (≈ 1240 cm^{-1}), and others <1115 cm^{-1} *(1b,c, 3a, 4c, 12, 14, 17)*.

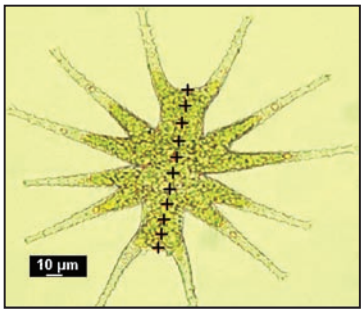

Figure 7. *Micrasterias hardyi*; the cross marks indicate 10 µm diffraction limit steps of SR-FTIR microspectroscopy spectra (*15*, used with permission).

As shown in Figure 7, several microalgae cells, *Micrasterias hardyi*, were divided into two groups, one control and one that was exposed to changes in phosphorus (P) levels. A series of image maps generated by SR-FTIR microspectroscopy was used to detect cellular biomolecular chemicals at diffraction limit increments of 10 µm. The SR-FTIR allowed researchers to observe while the microalgae cells were in the process of making chemical responses to changes in P levels. The experimental and control cells thrived, even after both groups were exposed for days to SR-FTIR photons. The concentrations of cellular components differed according to P exposure, making it apparent that the cells were altered only by P changes, not by SR-FTIR exposure. SR-FTIR microspectroscopy can be successfully used to identify in-process chemical changes in individual cells *(1b, 3a, 15, 16)*.

The studies regarding Figures 6 and 7 indicate that SR-FTIR microspectroscopies are able to transmit photons through cell internal structures and discern fine compositional differences without harming the cell *(14, 15, 17, 21)*.

Human fingerprints are complex chemical mixtures originating from a micron-scale surface heterogeneous mix of organic molecules exuded by each person's fingertips. SR-FTIR is a sensitive enough tool to test such minute and complex organic molecular species. Experiments indicated that SR-FTIR was able to detect fine peak structure and that fingerprints from each person revealed distinctly different spectra. *(4a, 22)*

Experiment Summary

SR-FTIR is a very sensitive tool, ideal for investigating very small samples of IR-responsive species. These case studies strongly indicate that subjecting organic compounds, living cells, and living cellular colonies to SR-FTIR radiation, from hours to days, does not cause sample damage or alterations. The experiments herein have shown that SR-FTIR yields results of significant detail and quality, thus making traditional FTIR appear deficient by comparison. These studies indicate that SR-FTIR lacks problems with sample heterogeneity and is the only tool able to differentiate and identify highly complex chemical species in minute quantities *(1c, 3a,b, 4a,c, 5b, 13–17, 22)*.

Conclusion

Researchers may not be aware that conclusions derived from the potentially incomplete peak details of traditional FTIR may not be the same as those derived via SR-FTIR, or that samples may be needlessly ruined via traditional FTIR that would not have been altered via SR-FTIR. Notably, forensics and other fields must routinely rely on evidentiary data gathered from small, dilute, or irreplaceable samples. When low signal-to-noise ratios, such as those of traditional FTIR, obscure a sample's signal, wrong conclusions can be reached, possibly causing severe ramifications. However, SR-FTIR would likely lead to correct conclusions. Researchers must cogitate on the superior traits of a SR-FTIR (for samples that are small, dilute, scarce, where traditional FTIR gave deficient results, or where determining the presence of specific species or bonds is crucial) as compared to the better qualities of traditional FTIR (for samples that require immediate results). The potential value of revealing unknowns via SR-FTIR's unequalled detection capabilities need to be anticipated and then balanced against time and travel expenses associated with using a SR-FTIR. However, a researcher cannot use traditional FTIR and have the expectation of revealing information that may be obtainable only via a SR-FTIR.

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